

REMARKS/ARGUMENTS

Claims 1-16 are pending in the present application. Claims 1-4 and 6-7 have been amended without prejudice and without acquiescence. Claims 17-18 have been added. Applicants have attached as Appendix A a marked-up version showing the changes contained herein. For the convenience of the Examiner, Applicants have also attached as Appendix B a clean copy of the pending claims. No new matter has been added.

The issues outstanding in this application are as follows:

- Claims 1-16 were rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite.
- Claims 1, 3, 5, 7, 9 and 13 were rejected under 35 U.S.C. § 102(b) as being anticipated by Ronaghi et al. (Anal. Biochemistry, 1996).
- Claims 1-16 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Ronaghi et al., in view of Mian et al., (US 6,319,469).

Applicants respectfully traverse the outstanding rejections, and Applicants respectfully request reconsideration and withdrawal thereof in light of the amendments and remarks contained herein.

I. 35 U.S.C. § 112, second paragraph rejection

Claims 1-16 are rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite. Applicants respectfully traverse.

In order to advance the prosecution of the present application, Applicants have amended claims 1, 2, 3, 4, 6, and 7 to clarify the scope of the present invention without prejudice and without acquiescence. Support for the measurement of released pyrophosphate can be found on page 1, lines 24-33 and page 9, lines 11-30. Support for the “is complementary” can be found on page 4, lines 22-25. Support for the double stranded DNA comprising one strand of primer DNA and on strand of sample DNA can be found on page 3, lines 16-17. Support for the primers of one or more reaction area being hybridised to the sample DNA can be found on page 3, lines 16-29. Support for the required number of times

for the identification of said sequence can be found on page 1, lines 24-33; page 4, lines 9-13; page 8, lines. Support for attaching a nucleic acid to a surface can be found on page 5, lines 2-7; page 7, lines 17-34 and page 8, lines 14-19.

Thus, in light of the above arguments and amendments, Applicants respectfully request that the rejections be withdrawn.

II. 35 U.S.C. § 102(b)

Claims 1, 3, 5, 7, 9 and 13 are rejected under 35 U.S.C. § 102(b) as being anticipated by Ronaghi et al. (Anal. Biochemistry, 1996). The Action states that Ronaghi et al. teaches the methods of using a microfluidic device to determine a nucleotide base in a nucleic acid sample. Applicants respectfully traverse.

Anticipation of a claim is only established where “each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.” *Verdegel Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

The teachings employed by Ronaghi et al. does not anticipate the claims found in the present application. The Examiner erroneously construes the meaning of “microfluidic device” to mean any suitable device which holds liquids on a microliter scale. Applicants contend that it is well known to those of skill in the art that the term “microfluidics” and “microfluidic device” refer to a device in which there is a transport of liquid. Applicants refer the Examiner to passages of the present application that discusses microfluidic devices (see page 5, line 32 to page 6, line 8 and Figure 1). The microfluidic devices discussed require transport of sample reagents and the like by the use of liquid flow, e.g., from a sample loading or application area to one or more reaction chambers and a detection chamber.

Yet further, Applicants refer the Examiner to page 9 of the Action in which the Examiner states that Ronaghi et al. does not teach a method for identifying the sequence of a portion of sample DNA wherein the steps are performed in a microfluidic device. Thus, the Examiner has acknowledged later in the Action that Ronaghi et al. does not teach the use of a microfluidic device, thus, Ronaghi et al. can not anticipate the present invention.

Therefore, since the Ronaghi et al. does not teach a microfluidic device, Ronaghi et al. is precluded from anticipating the present claims. Thus, the rejection of claims 1, 3, 5, 7, 9 and 13 is improper, and withdrawal of the rejection is respectfully requested.

III. 35 U.S.C. § 103(a)

Claims 1-16 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Ronaghi et al. in view of Mian et al., (US 6,319,469). The Action states that Ronaghi et al. teaches a method of identifying a sequence of a portion of DNA, but does not teach identifying a sequence of a portion of DNA using a microfluidic device. The Action further states that Mian et al. teaches the use of a microfluidic device for the method of Ronaghi et al. Applicants respectfully traverse.

The MPEP sets forth the guidelines to establish a *prima facie* case of obviousness under 35 U.S.C. § 103 (MPEP § 2143.3). Three basic criteria must be met to establish a *prima facie* case of obviousness. The three criteria are:

- 1) a suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings;
- 2) a reasonable expectation of success; and
- 3) the prior art references must teach or suggest all the claim limitations.

In light of the above criteria, Applicants assert that the Office has not established a *prima facie* case of obviousness to reject the claims under 35 U.S.C. § 103. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438, (Fed. Cir. 1991). A *prima facie* case necessitates disclosure of the source for either a suggestion or motivation to modify a reference to produce the present invention, and a reasonable expectation of success of producing the present invention.

Applicants contend that neither Ronaghi et al. nor Mian et al. alone or in combination teaches and/or suggests the present invention. Ronaghi et al. teaches sequencing using a stepwise addition of various nucleotides that are added or not added to an extending primer. Mian et al. teaches DNA sequencing in Example 7 (column 48-column 49). The DNA sequencing taught by Mian et al. is enzymatic sequencing by the Sanger method, which

comprises extending the primer in simultaneous presence of all four nucleotides and trace amounts of a fluorescently labelled dideoxynucleotide, and ultimately uses electrophoresis to separate the dideoxynucleotide-terminated DNA fragments so that the sequence is determined by the fluorescence pattern of the DNA fragments. Thus, Mian et al. does neither teach the method of Ronaghi et al. nor the method of the present invention.

The present invention utilizes a microfluidic device for carrying out the sequencing reactions of Ronaghi et al. and accomplishes a number of advantages and circumvents a number of drawbacks. (See Page 2, lines 1-14, page 10, lines 14-19) Thus, by utilizing the microfluidic device of the present invention, excess reagents and soluble products can be easily removed/separated from a growing immobilized primer to decrease the risk of artifacts. Yet further, apyrase is not needed and the amounts of the reagents can be minimized without diminishing the reliability of the method.

A *prima facie* case of obviousness must be established by evidence rather than conjecture. *Ex parte Yamamoto*, 57 USPQ2d 1382, 1383, 1384 (CCPA 2000). In the present case, it is mere conjecture on the part of the Office that one of skill in the art would modify the methods of Ronaghi et al. and combine those methods with the apparatus of Mian et al. to result in the present invention. Thus, with the lack of teaching or suggestion to design a microfluidic apparatus that is capable of performing cycles of sequencing reactions in which each cycle is represented by a separate step comprising addition of deoxynucleotide, deoxynucleotide analogue or a dideoxynucleotide, Applicants assert that the references do not meet the basic requirements of a *prima facie* case of obviousness.

Yet further, Applicants contend that the level of skill in the art can not be relied upon to provide the suggestion to combine references. For example, a statement that modifications of the prior art to meet the claimed invention would have been "well within the ordinary skill of the art at the time the claimed invention was made" because the references relied upon teach aspects of the claimed invention is not sufficient to establish a *prima facie* case of obviousness without some objective reason to combine the teachings of the references. *Ex parte Levengood*, 28 USPQ2d 13001B. Pat. App. & Inter. 1993.

CONCLUSION

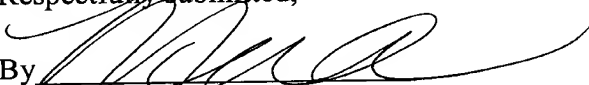
Claims 1-16 are pending in the present application. Claims 1-4 and 6-7 have been amended without prejudice and without acquiescence to clarify the scope of the present invention. Claims 17-18 have been added. No new matter has been added.

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 06-2375, under Order No. 10104789 from which the undersigned is authorized to draw.

Dated: January 16, 2003

Respectfully submitted,

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Appendix A
Version With Markings to Show Changes Made

1. (Twice Amended) A method of determining a nucleotide base in a nucleic acid sample comprising the steps of:

- (i) incubating the nucleic acid sample with a primer, DNA polymerase, and a deoxynucleotide triphosphate, deoxynucleotide triphosphate analogue or a dideoxynucleotide triphosphate, which releases pyrophosphate when added to the primer by action of DNA polymerase;
- (ii) measuring the pyrophosphate released in step (i); and
- (iii) ~~identifying~~determining that the nature of the nucleotide base is complementary to said deoxynucleotide triphosphate, deoxynucleotide triphosphate analogue or dideoxynucleotide triphosphate that is incubated in step (i), added by
~~measuring which nucleotide caused the release of pyrophosphate in step (ii)~~

wherein steps (i) to (iii) are performed in a microfluidic device.

2. (Twice Amended) A method for identifying the sequence of a portion of sample DNA comprising the steps of:

- (i) forming immobilised double stranded DNA comprising one strand of sample DNA and one strand of primer DNA on one or more reaction areas in a microchannel structure of a microfluidic device, DNA primers of said one or more reaction areas are hybridised to said sample DNA;
- (ii) adding a deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide and a DNA polymerase to each of said one or more reaction areas so that extension of primer ~~only occurs if there is~~ a result from a complementarity of the added deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide with the strand of sample DNA that is part of the immobilized double stranded DNA; and

- (iii) detecting whether or not the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide added in step (ii) ~~has been~~is added to the primer DNA in said one or more reaction areas;
- (iv) repeating steps (ii) and (iii) ~~as required with a different deoxynucleotides, deoxynucleotide analogues or dideoxynucleotides~~ the required number of times for the identification of said sequence; and
- (v) identifying said sequence from the results of step (iii).

3. (Twice Amended) A method of determining a nucleotide base in a nucleic acid sample comprising the steps of:

- (i) attaching 0.1 – 200 pmol of a primer DNA or single stranded sample DNA ~~sample~~ to each of between one and 100,000 pre-determined areas ~~on~~ within the surface of a microfluidic device;
- (ii) hybridising ~~small amounts of~~ single stranded sample DNA to said primer DNA or primer DNA to said single stranded sample DNA respectively to each of the predetermined areas by utilizing said primer DNA or said single stranded sample DNA that is attached to said predetermined areas in step (i);
- (iii) adding a deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide and a DNA polymerase so that extension of the primer ~~only occurs~~ by action of said DNA polymerase with consequent release of pyrophosphate, ~~if there is~~ as a result from a complementarity for the added deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide with the sample DNA; and
- (iv) measuring the release of pyrophosphate and from which predetermined area ~~on the device it is released; and~~ thereby enabling determination of said nucleotide base.
- (v) ~~repeating steps (iii) and (iv) as required to construct a DNA sequence for the elongated primers, and hence for portions of the sample DNA.~~

4. (Twice Amended) A method for identifying the sequence of a portion of sample DNA, comprising the steps of:

- (i) adding sample DNA to a predetermined area on a microfluidic device;
- (ii) moving the sample DNA to a reaction chamber on the microfluidic device;
- (iii) attaching the sample DNA to a surface of the reaction chamber, wherein a DNA primer is hybridised to the sample DNA in a single stranded form, or hybridising the sample DNA in single stranded form to a DNA primer that is attached to the surface of the reaction chamber~~primer is hybridised to the DNA;~~
- (iv) extending the primer in the presence of a DNA polymerase with a deoxynucleotide, deoxynucleotide analogue, or dideoxynucleotide, wherein the extension is indicated by detection-release of pyrophosphate~~released~~ from the extension reaction; and
- (~~vi~~iv) repeating step (iv) the required number of times for the identification of said sequence;
- (v) identifying said sequence from the deoxynucleotides, deoxynucleotide analogues, or dideoxynucleotides that resulted in primer extension in step (iv).
~~as required to establish the sequence of the extended primer.~~

6. (Twice Amended) The method of claim 2, wherein ~~the detection step involves labeled terminators~~ a dideoxynucleotide which is labelled is added in step (ii).

7. (Twice Amended) The method claim 1, wherein the detection of the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide ~~incorporation~~ added to the primer is performed in real time.

Appendix B
Claims pending as of January 16, 2003

1. (Twice Amended) A method of determining a nucleotide base in a nucleic acid sample comprising the steps of:

- (i) incubating the nucleic acid sample with a primer, DNA polymerase, and a deoxynucleotide triphosphate, deoxynucleotide triphosphate analogue or a dideoxynucleotide triphosphate, which releases pyrophosphate when added to the primer by action of DNA polymerase;
- (ii) measuring the pyrophosphate released in step (i); and
- (iii) determining that the nucleotide base is complementary to said deoxynucleotide triphosphate, deoxynucleotide triphosphate analogue or dideoxynucleotide triphosphate that is incubated in step (i),

wherein steps (i) to (iii) are performed in a microfluidic device.

2. (Twice Amended) A method for identifying the sequence of a portion of sample DNA comprising the steps of:

- (i) forming immobilised double stranded DNA comprising one strand of sample DNA and one strand of primer DNA on one or more reaction areas in a microchannel structure of a microfluidic device, DNA primers of said one or more reaction areas are hybridised to said sample DNA;
- (ii) adding a deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide and a DNA polymerase to each of said one or more reaction areas so that extension of primer occurs as a result from complementarity of the added deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide with the strand of sample DNA that is part of the immobilised double stranded DNA;

- (iii) detecting whether or not the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide added in step (ii) is added to the primer DNA in said one or more reaction areas;
- (iv) repeating steps (ii) and (iii) with different deoxynucleotides, deoxynucleotide analogues or dideoxynucleotides the required number of times for the identification of said sequence; and
- (v) identifying said sequence from the results of step (iii).

3. (Twice Amended) A method of determining a nucleotide base in a nucleic acid sample comprising the steps of:

- (i) attaching 0.1 – 200 pmol of a primer DNA or single stranded sample DNA to each of between one and 100,000 pre-determined areas within the surface of a microfluidic device;
- (ii) hybridising single stranded sample DNA to said primer DNA or primer DNA to said single stranded sample DNA, to each of the predetermined areas by utilizing said primer DNA or said single stranded sample DNA that is attached to said predetermined areas in step (i);
- (iii) adding a deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide and a DNA polymerase so that extension of the primer occurs by action of said DNA polymerase with consequent release of pyrophosphate as a result from complementarity for the added deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide with the sample DNA; and
- (iv) measuring the release of pyrophosphate and from which predetermined area it is released thereby enabling determination of said nucleotide base.

4. (Twice Amended) A method for identifying the sequence of a portion of sample DNA, comprising the steps of:

- (i) adding sample DNA to a predetermined area on a microfluidic device;

- (ii) moving the sample DNA to a reaction chamber on the microfluidic device;
- (iii) attaching the sample DNA to a surface of the reaction chamber, wherein a DNA primer is hybridised to the sample DNA in a single stranded form, or hybridising the sample DNA in single stranded form to a DNA primer that is attached to the surface of the reaction chamber;
- (iv) extending the primer in the presence of a DNA polymerase with a deoxynucleotide, deoxynucleotide analogue, or dideoxynucleotide, wherein the extension is indicated by release of pyrophosphate from the extension reaction;
- (v) repeating step (iv) the required number of times for the identification of said sequence; and
- (vi) identifying said sequence from the deoxynucleotides, deoxynucleotide analogues, or dideoxynucleotides that resulted in primer extension in step (iv).

5. The method of claim 1, wherein the pyrophosphate release is detected by light emitted from a luciferin luciferase reaction.

6. (Twice Amended) The method of claim 2, wherein a dideoxynucleotide which is labelled is added in step (ii).

7. (Twice Amended) The method claim 1, wherein the detection of the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide added to the primer is performed in real time.

8. The method of claim 1, wherein the microfluidic device is a disc and the fluids are moved by centripetal force.

9. The method of claim 3, wherein the pyrophosphate release is detected by light emitted from a luciferin luciferase reaction.

10. The method of claim 4, wherein the pyrophosphate release is detected by light emitted from a luciferin luciferase reaction.

11. The method claim 2, wherein the detection of the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide incorporation is performed in real time.

12. The method of claim 2, wherein the microfluidic device is a disc and the fluids are moved by centripetal force.

13. The method claim 3, wherein the detection of the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide incorporation is performed in real time.

14. The method of claim 3, wherein the microfluidic device is a disc and the fluids are moved by centripetal force.

15. The method claim 4, wherein the detection of the deoxynucleotide, deoxynucleotide analogue, dideoxynucleotide, or dideoxynucleotide analogue incorporation is performed in real time.

16. The method of claim 4, wherein the microfluidic device is a disc and the fluids are moved by centripetal force.

17. The method of claim 3, wherein steps (iii) and (iv) are repeated with a deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide.

18. A method for identifying a sequence of a portion of sample DNA comprising the steps of:

(i) forming immobilised double stranded DNA comprising one strand of sample DNA and one strand of primer DNA on one or more reaction areas in a microchannel structure of a microfluidic device, the primers of said one or more reaction areas are hybridized to said sample DNA;

(ii) adding fluorescently labelled dideoxynucleotides and a DNA polymerase to each of said one or more reaction areas so that extension of primer occurs as a result from complementarity of the added dideoxynucleotides with the strand of sample DNA that is part of the immobilised double stranded DNA;

(iii) detecting whether or not a deoxynucleotide added in step (ii) is added to the primer DNA in said one or more reaction areas;

(iv) repeating steps (ii) and (iii) with other fluorescently labelled dideoxynucleotides; and

(v) identifying said sequence from the result of step (iii).